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AN INTEGRATED PROCESS FOR PRODUCTION OF ETHANOL

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ABSTRACT

Although many processes for production of ethanol from lignocellulosic materials have been proposed during the last thirty years, there are no processes that appear to be competitive from an economic standpoint at this time. Major problems are in the areas of hydrolysis of cellulose to fermentable sugars, ethanol inhibition during fermentation, recovery of anhydrous ethanol, and recovery and recycle of active yeast and enzymes. In July, 1987, a project was initiated at Texas Tech under subcontract to the Solar Energy Research Institute, to address this last problem, namely that of recovery of active yeast and cellulase from the fermenter effluent in a SSF process.

Four major tasks will be performed under this subcontract. First a thorough literature review will be conducted to identify as many possible methods for achieving the desired separation as possible. Following this, a series of preliminary experiments will be conducted to obtain physical as well as chemical properties of the yeast-enzyme-cellulose system. These data will be used to propose a separation scheme which will be studied in the fourth phase of the project through extensive experimental studies and modeling of the process.

The current status is that the literature review is nearly complete and key process variables have been identified for the experimental phase of the work. The preliminary research plan and direction is reported in this review although the exact plan has not been completed at this time. The research will focus on factors which affect the adsorption/desorption of cellulase and its individual components in the presence of crystalline cellulose.

AN INTEGRATED PROCESS FOR PRODUCTION OF ETHANOL

INTRODUCTION

A very promising technique for production of ethanol from lignocellulosic materials involves simultaneous saccharification and fermentation (Gauss, 1976, and Takagi, 1977). In this process, cellulase enzymes and the fermenting yeast are introduced in a single vessel along with the cellulosic substrate with the goal of direct production of ethanol. This process has the advantage of alleviating the inhibition of the enzyme by consumption of cellobiose and glucose as it is produced. Other investigators have evaluated this process and have demonstrated a strong potential for economic success (Wyman, 1986). The development of this step in the process shows great potential for commercialization of ethanol production from cellulose, but at least two major questions still remain to be answered. The first involves recovery of ethanol and the second involves the recovery of cellulase and yeast.

In the proposed process described below, ethanol recovery is achieved through vacuum fermentation similar to the Vacuferm process as reported by Ramalingham and Finn in 1977, and Cysewski and Wilke in 1977. Vacuum fermentation has the advantage of reducing ethanol inhibition in the fermenter, as well as removing ethanol from the fermentation broth at low temperatures. This should prevent denaturation of the enzyme and enhance its recovery and recycle. The second problem mentioned above, that of enzyme recovery for recycle is addressed in this project. A more detailed description of the problems involved are presented in the literature review below.

DESCRIPTION OF PROPOSED PROCESS

As stated previously, the proposed process is simultaneous saccharification and fermentation under vacuum. A block flowsheet of the proposed process is shown in Figure 1. A description of each major process step and the qualitative advantages of these steps are given below.

Biomass (lignocellulosic material) is ground to a small particle size. It is anticipated that a particle size of -10 mesh will be sufficient, but it may be necessary to use a smaller size. A disk attrition mill or ball mill is used for this step. The ground biomass is fed by a screw conveyor to the SSF vessel. If sterilization of the biomass is necessary, it can be accomplished by steam injection in the auger. This will not only sterilize the feedstock, but will enhance wetting of the material.

Recycle yeast and cellulase are added to the SSF vessel along with recycle solids, makeup water, makeup cellulase and either acid or base to adjust the pH to the desired level. This reactor will be operated as a

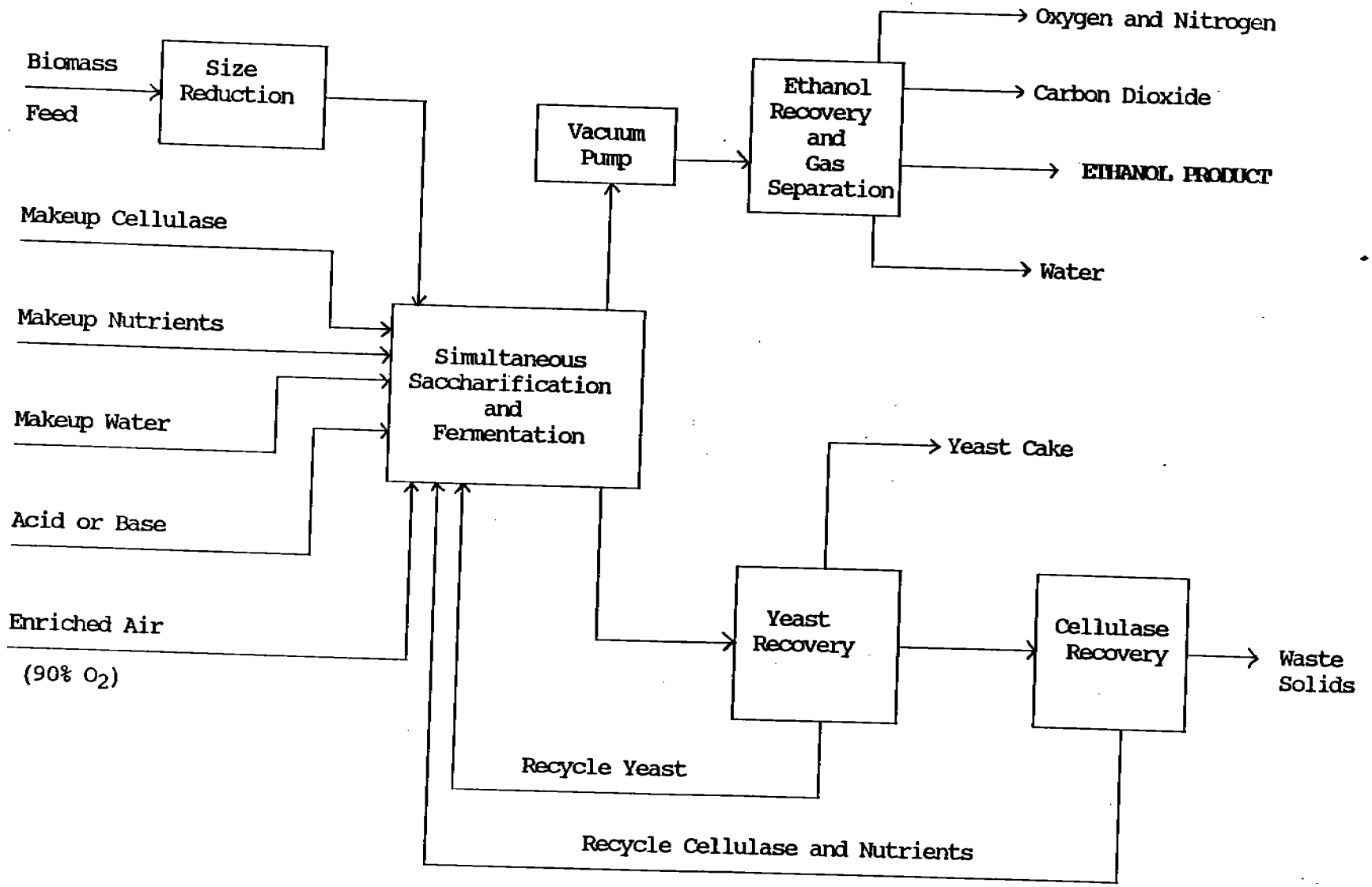

Figure 1. Block Flowsheet of Proposed Vacuum SSF Process

continuous stirred tank (CSTR) at a pH between 4.5 and 5.0 and temperature of 35 to 45 °C. These conditions should provide reasonable activity for the cellulase and fermenting yeast. This vessel is designed to operate at relatively low conversions per pass of both cellulose and glucose. The advantage of operating at a low conversion is that the reactor size will be small which will significantly reduce the capital cost of the process.

Because this is a CSTR, the concentration of components in the broth will be constant. It is anticipated that the ethanol concentration will be in the range of 20 to 50 g/L which will minimize ethanol inhibition, but provide sufficient driving force for concentrating ethanol in the vapor product from the fermenter. The glucose concentration should be in the range of 30 to 50 g/l so that it is not a growth limiting nutrient (Boyd, 1985), but is low enough to prevent significant inhibition of hydrolysis. This glucose concentration is lower than the optimum of 100 g/L reported by Cysewski and Wilke (1976).

Some oxygen is necessary for the fermentation and must be relatively pure. In order to minimize the operating cost, enriched air will be used instead of pure oxygen. The Prism separator, marketed by Permea, Inc., can be used to provide a 90% oxygen, 10% nitrogen mixture at a low cost (Kurz, 1986). It is proposed this be the source of oxygen for sparging the fermenter. Based on the results of Cysewski and Wilke (1976), the fermenter will be operated at an oxygen tension of 0.05 to 0.10 mm Hg.

A vacuum pump will be used to remove the vapors from the fermenter. The vapor will contain ethanol, water, carbon dioxide, oxygen, and nitrogen. Ethanol and water will be removed from this stream by condensation. Because of the salts present in the fermentation broth, the ethanol concentration in the vapor will be fairly high (Fan, 1984). It is anticipated that the condensed ethanol-water mixture will contain approximately 200 g/L of ethanol (Maiorella et al., 1979).

The slurry effluent from the fermenter contains unreacted biomass, yeast, ethanol, glucose, nutrients, and cellulase. The major objectives of this research will be to develop a scheme to separate this mixture for recovery and recycle of yeast, active cellulase, and nutrients to the SSF vessel. This is explained further in the research plan.

A preliminary material balance was performed on this process. The feedstock was wheat straw containing 45% by weight cellulose (Anderson and Anderson, 1980). Major assumptions regarding conversions and other operating factors are shown below.

1. Cellulase required for hydrolysis = 30 IU/g glucose produced (Wilke et al., 1983).
2. Cellulose conversion per pass = 50% (Lee and Fan, 1983).
3. Oxygen tension in fermenter = 0.07 mm Hg (Wilke et al., 1981).

4. Glucose conversion in fermenter = 70%.
5. Ethanol yield = 0.42 kg/kg glucose fermented (Ramalingham and Finn, 1977).
6. Carbon dioxide yield = 0.42 kg/kg glucose fermented (Ramalingham and Finn, 1977).
7. Moisture content of filter cake = 25% (Ulrich, 1984).
8. Moisture content of yeast cake = 30% (Ulrich, 1984).
9. Recycle 50% of unreacted solids from filter cake.
10. Ethanol recovery from fermenter vapor = 98%.

Using these assumptions, ethanol yield from hydrolysis and fermentation of wheat straw is 20.5 gal/ton. This compares favorably with the ethanol yield of 24 gal/ton of corn stover reported by Wilke et al. (1981).

There are many technical uncertainties in the process described above. The research plan presented below is designed to provide preliminary answers to the key unknowns so that a preliminary design and economic evaluation can be performed on the process to determine if further research is warranted.

RESEARCH OBJECTIVES

A key question affecting the economic feasibility of this process is whether or not active yeast and cellulase can be recovered from the fermentor effluent. This project is designed to address this question. The tasks to be performed under this subcontract are shown below.

Task I -The subcontractor shall conduct a thorough literature survey on the separation of cellulase enzymes and yeast cells from cellulose and cellulosic residues. A brief summary of this review shall be prepared and submitted to the project monitor at the completion of this task along with a recommendation of the research approach for TASK II. Approval of this approach shall be required before initiating TASK II.

Task II -Preliminary experimental data shall be obtained on key properties that are critical to the separation approach to complement data found in TASK I. These experiments shall be performed using Genencor 150L cellulase, S. cerevisiae and B. clausenii yeasts, and Sigmacell 50 cellulose. Data shall be collected on terminal velocities of yeast cells and cellulose particles for evaluation of gravity separation of these components. Preliminary adsorption isotherms at varying temperatures and pH shall be obtained for the various enzymes in the cellulase system. Particular attention shall be paid to the fate of cellobiohydrolase in relation to the glucanases.

Task III -Based on the results from TASKS I and II and with the approval of the project manager, the most promising separation scheme(s) shall be selected for evaluation. A detailed plan shall be proposed detailing the

research approach to be followed. This plan shall be approved by the technical monitor before beginning TASK IV.

Task IV - Detailed experimental data shall be obtained for the separation scheme selected for synthetic mixtures of cellulose, cellulase, lignin, and yeast. This data shall include both overall and component material balances and shall follow the approach agreed to in TASK III.

LITERATURE REVIEW

Several studies have been conducted to address the equilibrium between adsorbed and desorbed cellulase in the presence of cellulose. Few of these studies have specifically reported adsorption isotherms for the systems as a function of key process variables. There is also conflicting information in the literature as to the effect of some process variables such as temperature and pH.

Riaz and Wilke (1978) reported that adsorption of the cellulase proteins increases as temperature increases, which is the opposite that one would expect for most adsorption situations. This is a relatively old reference, but Wilke's group has continued work in this area. The pertinent theses and other reports have been ordered but have not yet been received for review.

The effectiveness of pH adjustment in desorption of the cellulase is somewhat uncertain at the present time. Riaz and Wilke (1978) evaluated the desorption of enzyme from cellulose over a pH range of 2-12. They report that at a pH of 10, only 12-14% of the original enzyme activity was recovered. On the other hand, Beldman, et al (1984) state that "our unpublished results and those of others indicate that adsorbed cellulase can be recovered by a pH shift to the alkaline region (pH 10) or by the use of surfactant." In this statement, they refer to a paper by Reese (1982) that reports the use of pH adjustment to 10 to recover the active enzyme. This paper has been ordered, but has not yet been received. It is apparent from the conflicting information on the effect of pH that an investigation of adsorption isotherms as a function of both temperature and pH must be conducted during the course of this research.

Several studies have been identified in which a chemical agent, or surfactant, was used to either prevent adsorption or enhance desorption of the enzyme from the cellulose substrate. Riaz and Wilke (1978), Castanon and Wilke (1981), and Rao, et al. (1983), all report that the surfactant Tween 80 is effective in preventing adsorption or enhancing desorption of the enzyme from spent cellulose. While this is a promising result, the cost effectiveness of a surfactant is uncertain, as well as the mechanism by which it operates. In the research to be performed under this subcontract, it is not anticipated that the use of surfactants, or other agents such as urea (Riaz and Wilke, 1978), will be investigated in any depth. From an economic

standpoint, it may not be desirable to achieve an extremely high percentage recovery of the cellulase if the cost of that incremental recovery will be prohibitive.

Gusakov, et al. (1985), presented a mathematical model for enzymatic hydrolysis of cellulose. They reported that enzyme inactivation occurs through three primary mechanisms. These mechanisms are; inactivation due to temperature, due to intensive stirring at the air-solution interface, and due to strong adsorption of the enzyme on the cellulose surface. They report that inactivation due to adsorption was reversible by adjusting the temperature or the pH. In another study, Tanaka, et al. (1986) reported results on the mechanism of cellulase adsorption during hydrolysis of crystalline cellulose. This article has just been received and appears to provide additional direction in planning this research. They do state that there is some irreversible adsorption inside the cellulose fibril and investigation of this mechanism is continuing.

The initial literature review is nearing completion. We are still awaiting two or three key publications, dealing with the effect of pH on the adsorption equilibrium and studies of the mechanism of adsorption.

PRELIMINARY EXPERIMENTAL STUDIES

Following completion of the literature review, a series of preliminary experiments will be conducted to identify the effect of key properties that are felt to be critical to the separation of the effluent from the SSF vessel. These preliminary experiments will be designed to identify the most promising schemes for recovery of active yeast and active cellulase for recycle in the process. At this time, it appears that the variables that will be examined in a preliminary sense involve settling velocities of yeast cells and cellulose particles for potential gravity separation to recover yeast. There may also be one or two filtration tests of yeast cells to obtain physical properties necessary to design a filtration system for yeast harvesting.

Most of the preliminary experiments will be focused on the adsorption isotherms for the system containing cellulase enzyme components and crystalline cellulose. A completely randomized experimental design with factorial treatment combinations will be used to study the range of variables shown in Table 1.

Table 1. Process Variable Study

Variable	Range
Contact time	5 - 60 minutes
Enzyme/Cellulose ratio	0.75 - 2 IU/g
pH	2 - 12
Temperature	0 - 50 °C
Glucose concentration	2 - 12 wt. %
Ethanol concentration	2 - 8 vol. %

The adsorption equilibrium will be determined by placing a known amount of crystalline cellulose in a solution of cellulase. The solution will be filtered and free enzyme activity in the filtrate determined. The amount of adsorbed enzyme will be determined by difference. Equilibrium will be determined for total enzyme activity, component activity, and total protein. This study will help identify the potential for recovery of adsorbed cellulase.

SUMMARY

There are no definitive results from this project as yet. It is anticipated that the preliminary experiments will be completed by mid-January, 1988.

ACKNOWLEDGEMENTS

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ECONOMIC EVALUATION OF PRETREATMENT AND NON-PRETREATMENT VENDOR SIZE TESTS AND COSTS FOR COMMERCIALIZATION OF THE CONTINUOUS ENZYMATIC SSF FOR PRODUCTION OF ETHANOL FROM CELLULOSE WASTE

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ABSTRACT

A process, owned by the University of Arkansas and called the UA process, biologically converts cellulosic materials (such as RDF) into ethanol. This process has been technically proven in both bench-scale tests and a 1 ton per day pilot plant, and now the UA process needs to be further developed so it can be commercially integrated into an overall waste-to-energy plant which converts raw MSW into recycable fractions (ferrous, glass, and aluminum), power, ethanol, and an animal feed co-product.

A 3-Phased program has been prepared by CADCO, Inc. to advance this process to the stage at which a commercial plant could be constructed and venture financing secured without burdening investors with unreasonable risk.

Phase I - Vendor Pretreatment Screening

Phase II - Extended Pretreatment Equipment Testing for Optimization Followed by a Revision of the Overall Plant Design and Economic Reassessment

Phase III - Design, Construction, and Operation of the 50 TPD Unit